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Effects of Seed Treatment Fungicides on *Ascochyta pinodes* of Field Pea Under Controlled and Field Conditions

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ABSTRACT

Nine fungicides namely thiram, chlorothalonil, metalaxyl, benomyl, thiabendazole, carbendazim, thiophanate-methyl, benalaxyl and iprodione at the rate of 0.001, 0.01, 0.1, 1.0, 10 g/L medium ai were tested *in vitro* against *Ascochyta pinodes* (teleomorph = *Mycosphaerella pinodes*) from field pea for inhibition of mycelial growth. Tests were also conducted on the effect of these fungicides on seed mycoflora at the rate of 2g ai/kg seed. Further studies were carried out on the effects of selected fungicides on seedling infection in growth chamber and field conditions.

Radial growth of *A. pinodes* culture was completely inhibited by carbendazim and thiabendazole at the lowest concentration tested (0.001 g/L), benomyl at 0.01 g/L, thiram, thiophanate-methyl and iprodione at 0.1 g/L. Other fungicides affected the growth at various degrees. Seed treatment with carbendazim, chlorothalonil and iprodione completely inhibited the recovery of *A. pinodes* from treated seeds while 2 to 3% incidence was obtained for thiram, benomyl and thiabendazole. Untreated seeds showed 16% incidence while benalaxyl and metalaxyl gave 15 and 12% incidence, respectively.

Seedling infection in growth chamber was completely controlled by carbendazim and iprodione and a reduction of 4.6% by chlorothalonil.

In field trial, seed treatment with fungicides didn't affect emergence date while there was significant difference ($p = 0.05$) due to variety. However, carbendazim and iprodione had high emergence on all the three varieties and significantly ($p < 0.05$) reduced the incidence of Ascochyta infection at both locations at early development period of the crop. Infection appeared in late July and a mean incidence of was 0.03, 3.6, 7.6 and 97.1% on 28 July, 5 August, 12 August and 19August at Denbi, respectively. But at Holetta, the incidence was 0.04, 4.0 and 96.9% on 28 July, 5 August and 12 August, respectively. At both locations, a fast increase from about 10 to 100% of incidence was observed within a week time, but at different weeks. Blight severity was slightly affected at the beginning of the season and became similar soon as the season progressed. A mean seed yield of 2.15 t/ha was obtained at Holetta while it was only 0.80 t/ha at Denbi that were significantly ($p < 0.05$) different. This was due to difference in blight pressure. Generally, treating seeds with carbendazim improved seed yield by 13.2% and with iprodione by 12.5% over the untreated control. Seed treatment with fungicides could be used as a component of integrated blight management in field pea production.

Key words: *Ascochyta pinodes*, fungicide, seed treatment, seedling infection, field pea, *Pisum sativum*

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INTRODUCTION

Ascochyta blight is among very important field pea diseases throughout the world (Lawyer, 1984) and is caused mainly by *Ascochyta pinodes* (Berk. & Blox.) Jones [teleomorph = *Mycosphaerella pinodes* (Berk. & Blox.) Vestergrn], in which this pathogen is the most destructive component of Ascochyta disease " complex" of field pea in Ethiopia (Gorfu and Beshir, 1994). It often causes serious yield and quality losses that were mostly dependent on time and level of infection, host reaction and prevailing local climatic conditions (Bretag *et al*., 1995b; Nasir and Hoppe, 1998). In Ethiopia, a mean seed yield loss of 31% rising to about 53% was reported (Gorfu, 2000) warranting a control measure. This pathogen, *A. pinodes*, affects all stages of field pea crop by decreasing plant growth, biomass, and ultimately the seed yield that were mostly reflected on seed weight and number of seeds per plant (Garry *et al*., 1998).

Blight symptoms are characterized by discrete brown to black flecks and undefined lesions on leaves, petioles, stems and pods that latter coalesce to form dark black large lesions and blighted foliage of the crop (Beasse *et al*., 1999). At seedling stage, infection from seed source take place mostly at the seed attachment place, with deep black discoloration advancing to the roots and then to the stems (Gorfu and Sangchote, unpublished data), which latter develops to the aerial parts of the plant. Severe infection at the soil level can some times girdle seedlings thereby leading to plant death (Nasir and Hoppe, 1998).

Ascochyta pinodes is seedborne pathogen where inoculum infecting and adhering on seed surface as dormant mycelia, spores and fruiting bodies of the fungus, could be responsible for the transmission of infection from seed to seedling (Bretag *et al*., 1995a; Maude, 1996). This infection on seedling ultimately establishes disease in a new crop (Moussart *et al*., 1998) where secondary spread may leads to blight epidemics.

Disease pressure on a crop in the field is the result of disease establishment and subsequent outbreak where seedborne pathogens are of special concern to producers due to the risk of introducing pathogen to new field or area (Mathur, 1995). Particularly infected seed used for planting would facilitate the establishment phase of a disease when the pathogen is seed transmitted like that of *A. pinodes*. In the same way, the primary inoculum of *A. pinodes* on field pea seed used for planting should be eradicated in order to reduce the risk of blight epidemics in new field pea crop. Seed treatment with fungicide is, therefore, one feasible alternative to eradicate the primary inoculum to avoid the risk of epidemics as Agarwal and Sinclair (1997) also emphasized.

A. pinodes is a polycyclic disease where many cycles of spores are produced and continuous infection occurs in the life span of a crop, and thus, very small amount of initial inoculum could probably initiate a serious epidemics**.** Some research evidences are reported in this regard on *Ascochyta* blight of field pea.

Michall *et al.* (1998) showed that the relationship of seed infection to disease pressure in the field and reported that inoculum on field pea seed was important source of *Ascochyta* blight. Moussart *et al.* (1998) also reported that seed infection played a significant role in the epidemiology of *Ascochyta* blight of field pea.

Many of the systemic fungicides available at present have the advantage and capacity of ease of application and increased efficacy as seed treatment options. Maude (1983) emphasized that fungicides used as seed treatment should penetrate the tissue of the seed and eliminate deep-seated infections of pathogens without causing phytotoxicity. From this one can understand that a fungicide and a target pathogen should come in contact or close enough to achieve effective control. Hence, *in vitro* sensitivity test could be one good alternative method to see the efficacy of fungicides

against the target pathogen, *A. pinodes*, prior to field experimentation.

Not many studies were made to determine the efficacy of fungicides under controlled conditions that considers direct contact of target pathogen with intended fungicide and as seed treatment against *A. pinodes* under field conditions. However, these are reports on the increased plant stand (Kraft, 1982), eradication of seedborne Ascochyta inoculum (Maude, 1966) and increased seed yield (Nasir and Hoppe, 1998) in field pea. On chickpea and lentil, Ascochyta blight is effectively controlled by seed treatment (Agarwal and Sinclair, 1997). And earlier studies of blight control in Ethiopia were restricted to variety screening and foliar applications of fungicides, but the utility of fungicide seed treatment can be assessed if information in the efficacy of fungicide in this regard is available.

Therefore, this paper reports the results of successive studies conducted to evaluate nine fungicides for their inhibition of mycelial growth of *A. pinodes* and their effects on seed mycoflora when used as seed treatment. Furthermore, it reports the efficacy of three selected fungicides in controlling seedling infection under controlled conditions and efficacy of two selected fungicides under field conditions. This is in an attempt to select a potential seed treatment fungicide against *A. pinodes* of field pea.

MATERIALS AND METHODS

1. Effects of fungicides on mycelial growth of *Ascochyta pinodes*

Nine fungicides namely thiram, chlorothalonil, metalaxyl, benomyl, carbendazim, thiabendazole, thiophanate-methyl, benalaxyl and iprodione were studied by incorporating fungicides into autoclaved Coon's agar. This medium was used because the test fungus *Ascochyta pinodes* and its teleomorph *Mycosphaerella pinodes* grow and sporulate very well on this medium. Coon's agar contains 4 g maltose, 2 g potassium nitrate, 1.2 g magnesium sulfate, 2.7 g potassium di-hydrogen orthophosphate and 20 g agar in one liter of distilled water (Bretag *et al.*, 1995a). The medium was autoclaved for 20 minutes at 121°C and cooled to 55°C in water bath, then the required fungicide concentration was added. Each fungicide product was tested at five concentrations viz. 0.001, 0.01, 0.1, 1.0, and 10 g active ingredient (ai) per liter medium and unamended medium was included as a control. All the fungicides tested were wettable powder formulations.

Pure culture of *A. pin*odes was isolated from naturally infected seeds of a field pea (variety Tegegnech) obtained from the Holetta Agricultural Research Center (Ethiopia) where fungicides were not regularly applied. It was purified by repeated sub culturing from the tips of actively growing hyphe of the fungus on the same medium, Coon's agar.

Inhibition of mycelial growth was evaluated by transferring an agar disc of 5mm diameter from actively growing edges of new colony (7-day-old) to the center of fungicide amended medium or a control medium. The source culture was transferred to the center of test petri dishes by placing one disc upside down to insure the close contact of the test fungus and the fungicide in the medium.

The experiment was designed in CRD with 4 replications and a petri dish representing one replicate. All petri dishes were incubated at 23±1° C in the dark. Fungal growth was measured periodically from the third day till the whole petri dish was covered with the fungus on the control treatment. The percentage inhibition of mycelial growth by a fungicide was calculated by the formula: [1-(diameter of mycelial growth in the fungicide treated plate/diameter of mycelial growth in the untreated control)] X 100, according to Hwang and Kim (1995). Effective concentration (EC_{50}) causing 50% growth inhibition values were obtained for each fungicide from regression lines plotting percentage inhibition on probit scale versus

log concentration.

2. Effect of fungicides on seed mycoflora

The effects of all nine fungicides used in the growth inhibition experiment above were studied against seed mycoflora of field pea. Each fungicide was tested on naturally infected seed lot of variety Tegegnech having a mean seed infection of 12.9 ± 3.2%. The fungicides were tested at the rate of 2g ai per 1kg seed. The required amount of seed and fungicide was vigorously rotated in glass bottles with micro seed treating electrical machine for 30 minutes. The machine ensured uniform distribution of the fungicides on the seed coat of field pea seeds. The untreated control treatment was handled in the same way for all processes except fungicide. These treated seeds were subjected to standard blotter technique (Agarwal and Sincliar, 1997) for isolating the mycoflora of the seeds.

Ten seeds of each treatment were placed on four layers of moist filter paper in a 9cm-diameter petri dish. Hundred seeds were used in each treatment. The preparation is then incubated at 23±1°C under alternating period of 12-h near-ultraviolate light (NUV) produced by Philips black light lamps (fluorescent 40W) and 12-h darkness for subsequent days to encourage fructification. After 10 days of incubation fungi on each seed were recorded and identified. Germination of the seed was also counted. The data obtained were subjected to statistical analysis following the procedures described by Cody and Smith (1997).

3. Effects of selected fungicides on seedling infection under controlled conditions

The effect of three fungicides (selected based on the results of growth inhibition and seed mycoflora assay above) on seedling infection was studied in plastic pots with sand in growth chamber. The seed treatment was done as in Section 2 above (seed mycoflora experiment) on a seed lot with known infection level (12.9 ± 3.2%) determined by previous seed infection assay (Gorfu and Sangchote, unpublished data). Each treatment was set using 100 seeds in plastic pots with moistened sand. Five seeds per pot were seeded at the depth of about 3cm. They were incubated in growth chamber under a temperature regime of 20±1°C and alternating period of 12-h white lamp and near-ultraviolate light (NUV) produced by Philips black light lamps (fluorescent 40W) and 12-h darkness throughout the experiment period. Tap water was used to replenish soil moisture by adding about 30 ml every morning.

Emergence was monitored every day for two weeks. Emergence index (EI) was calculated according to Sordona (1978) with the following formula. EI = [(SN x D)/T] where N is number of seedlings emerged in 24h period and D is days after planting that seedling count was made; and T is total seedling emerged in each replication.

Three weeks after sowing, plant stand was recorded and expressed as percentage emergence. Then all plants were carefully removed and washed for infection assessment. Infection was scored for Ascochyta severity on each plant using 0-5 rating scale where 0=healthy and 5=a complete damage by the fungus as previously done by Phillips (1990). Plants were excised at seed attachment level where height was measured, fresh and dry weight of shoots and roots were recorded. Dry weight of the seedling was determined by oven dry method, which samples were dried at 115°C for 24 hours.

Infection of *A. pinodes* was confirmed through isolation of each infected parts of the seedling on Coon's agar. The data obtained was subjected to statistical analysis following the procedures described by Cody and Smith (1997).

4. Effects of seed treatment on Ascochyta blight development and seed yield of field pea under field conditions

Ascochyta infection and blight development, and ultimate seed yield were studied by planting treated seeds of three varieties using

two fungicides at two locations in Ethiopia in the 2002 crop season. The experiment was carried out at two contrasting locations (Holetta and Denbi) in rainfall and temperature regimes but having the same disease threat to field pea crops. The two locations have almost similar disease pressure caused by *Ascochyta pinodes*.

A factorial combination of 3 fungicides and 3 varieties was studied in RCBD with 4 replications and a plot size of 8 m^2. The three fungicide treatments were carbendazim, iprodione treated and the untreated seeds (control) while the three varieties were LocalH, Mohanderfer and Tegegnech having the same level of seed infection of 20.3 ± 2.7% with *A. pinodes*. The factorial combination of these factors constituted nine treatments.

The same seed rate, 150 kg/ha, was used for the three varieties. Seeds were divided in to 10 rows of 20 cm apart and 4 m long. Defined plots were allotted with 2 m of allay surrounding each plot. The soil of each plot was solarized for about 72 hours by covering with transparent polyethylene sheet where the temperature in the covered soil reached 62°C at Denbi and 53°C at Holetta. This was done to reduce the survival of Ascochyta inocula in soil.

Seed treatment was done by applying 2 g (ai) of test fungicide onto 1 kg of pea seeds in polyethylene bag. Then the seeds were vigorously shaken for 30 minutes to ensure uniform coating and distribution of the fungicides. The untreated control had received the same management except the fungicide.

In raising the crop, recommended agronomic practices were followed. Plots were planted with field pea seeds at the rate of 150 kg/ha on 21 June at Denbi and on 23 June at Holetta. Other agronomic practices including fertilizer rate, weeding, harvesting and threshing were followed in accordance to the recommendations made for the respective location. Three weeks after sowing, emergence count was done. Disease was monitored and assessed every week. Disease severity was assessed using percentage foliage damaged. The Area Under the Disease Progress was calculated according to Gorfu (2000).

At the end of the season, plant height, number of pods per plant and seeds per pod were counted on 10 randomly selected plants in each plot. After harvest, biomass and after threshing, seed yield and 100-seed weight were scaled. The data collected were subjected to statistical analysis through appropriate procedures described by Cody and Smith (1997).

RESULTS AND DISCUSSION

1. Effects of fungicides on mycelial growth of *Ascochyta pinodes*

Inhibition of radial growth of *A. pinodes* colony was greater at early stage (3 to 4 days) than in the last observation days (9 to 10 days) although the trend was the same and there were significant correlation ($r > 0.82$, $p < 0.003$) between values observed hence the result obtained on the tenth day was presented here. There were highly significant ($p \leq 0.01$) differences in radial growth among fungicides and their concentrations. Benomyl, carbendazim, iprodione, thiabendazole, thiophanate-methyl and thiram were highly toxic to *A. pinodes* (Table 1). Carbendazim and thiabendazole completely inhibited the radial growth at 0.001g/L, benomyl at 0.01g/L, while iprodione, thiophanate-methyl and thiram at 0.1g/L concentration. The EC_{50} values was 0.001 g/L for benomyl, <0.001 g/L for carbendazim, 0.003 g/l for iprodione, <0.001 g/L for thiabendazole, 0.04 g/L for thiophanate-methyl and 0.018 g/L for thiram after 10 days of incubation at 23°C in darkness (Table 1). EC_{50} value is the amount of fungicide needed to inhibit 50% growth of mycelia of *A. pinodes*, a parameter widely used to compare the toxicity of products.

Before this study, there had not been any report on the effect of these fungicides on mycelial

Table 1 Radial growth reduction (%) of *Ascochyta pinodes* on fungicide amended Coon's Agar and Effective Concentration (EC_{50}) of each fungicide used after ten days of incubation at 23°C in dark.

Fungicide	Radial growth reduction (%)					EC_{50} (g/l)
	Fungicide concentration (g/L medium)					
	0.001	0.01	0.1	1.0	10	
Benalaxyl	8.9	26.0	41.1	72.0	100.0	0.726
Benomyl	17.9	100.0	100.0	100.0	100.0	0.001
Carbendazim	100.0	100.0	100.0	100.0	100.0	<0.001
Chlorothalonil	20.0	35.0	58.2	100.0	100.0	0.075
Iprodione	40.3	93.4	100.0	100.0	100.0	0.003
Metalaxyl	0.0	0.0	0.0	15.5	91.0	>10
Thiabendazole	100.0	100.0	100.0	100.0	100.0	<0.001
Thiophanate-methyl	8.7	88.0	100.0	100.0	100.0	0.040
Thiram	4.1	61.8	100.0	100.0	100.0	0.018

growth of *A. pinodes* and our result clearly showed that those fungicides found effective in inhibiting radial growth would also affect different stages in the life cycle of *A. pinodes.* Kagorora and Griffiths (1994) also used amended media to check sensitivity of this fungus, *A. pinodes*, to many antioxidants. Berg *et al*. (2002) used the same method to evaluate six fungicides against *Alternaria cassiae* on cowpea seeds and Thomas and Sweetingham (2003) used this method to select seed treatment fungicides before field testing against lupine anthracnose fungus.

Nasir and Hoppe (1998) reported the use of thiram, iprodione, metalaxyl and thiabendazole alone or in combination to treat field pea seeds against several diseases including ascochyta blight. However in this study, among tested fungicides, carbendazim, benomyl, iprodione, thiabendazole, thiaphanate-methyl and thiram were sufficiently toxic to *A. pinodes* that would give basis for early selection of potential seed treatment fungicide. It is likely that fungicides having effect on mycelial growth could be also effective to control the disease in the field. The efficacy of seed treatment fungicides is partly influenced by the soil environment, in order to give sound recommendation the result of this study need to be verified under field conditions where many natural factors interact in field soils.

2. Effects of fungicides on seed mycoflora

The seed mycoflora of field pea was assessed on treated seed with fungicides using blotter method that is usually used in standard seed health testing. Nine fungi recovered at varying degree from fungicide treated seeds when incubated at 23°C (Table 2). These include *Ascochyta, Cladosporium, Alternaria, Curvularia, Aspergillus, Penicillium, Chaetomeum, Trichoderma* and *Monilia.* The most frequent was *Penicillium* followed by *Ascochyta, Cladosporium* and *Monilia* while the other fungi were at low frequency.

Fungicides such as carbendazim, ipridione, thiram, benomyl, thiabendazole and chlorothalonil were highly effective against most fungi including harmful pathogens such as *Ascochyta* and probably other fungi that could be antagonists. Thus practice

Table 2 Effects of fungicide seed treatment on seed mycoflora of field pea using standard blotter method.

Fungicide	*Ascochyta*	*Cladosporium*	*Alternaria*	*Curvularia*	*Aspergillus*	*Penicillium*	*Chaetomeum*	*Trichoderma*	*Monilia*	% Seed germination
Control (untreated)	16	8	3	3	1	48	1	1	5	99
Benalaxyl	15	5	0	1	2	36	0	0	0	100
Metalaxyl	12	3	2	0	9	45	0	0	0	96
Thiophanate-methyl	5	1	0	3	0	0	0	0	0	99
Thiabendazole	3	0	0	10	0	0	0	0	0	99
Benomyl	3	0	0	0	0	0	0	0	0	98
Thiram	2	0	0	0	0	0	0	0	0	77
Carbendazim	0	0	0	0	0	0	0	0	0	100
Iprodione	0	0	0	0	0	0	0	0	0	100
Chlorothalonil	0	0	0	0	0	0	0	0	0	99

of seed dressing to eradicate seed inocula and protect the young seedling from soilborne inocula at the same time reduce the soil mycoflora. Seed and soil mycoflora include many beneficial fungi like *Trichoderma* spp.

Among tested fungicides, carbendazim, chlorothalonil and iprodione completely inhibited *A. pinodes* in petri dishes on treated seeds while the untreated seeds (control) had 16% recovery. Benalaxyl and metalaxyl had a recovery of 15 and 12%, respectively, that was not significantly different ($p< 0.05$) from the control, hence ineffective for this purpose.

Fungicides kill or inhibit pathogenic fungi on the seed and in the soil near treated seeds (Hansing, 1978) and prevent or reduce disease establishment early in the season. In this study, three fungicides namely carbendazim, chlorothalonil and iprodione showed high efficacy against many seedborne fungi, although the test was done in petri dishes and the results suggesting their potential as seed treatment options field pea. The incidence of *A. pinodes* was zero for these three fungicides and the germination of field pea was also very high as fungicides applied and cause no phytotoxicity (Maude, 1983). In this study, germination of field pea seeds was severely affected by seed treatment with thiram and slightly with metalaxyl having 77 and 96% germination, respectively (Table 2). Nasir and Hoppe (1998) also reported 25% less emergence due to thiram treatment and this fungicide was less effective in controlling *M. pinodes*, which is the teleomorph of the same fungus, *A. pinodes*. The rest didn't affect germination and the untreated seeds had 99%, which was very high. This method was useful in detecting fungi other than *A. pinodes* that may be eradicated by the seed treatment. It also enabled us to observe fungicides having effects against wide spectrum of seed mycoflora if seeds could be treated before storage of short period between crop seasons. Shrestha *et al*. (2000) also used the same method to determine seed treatment options against *Alternatia* in two *Brassica* species and found that to be useful method before testing fungicides in the field.

3. Effects of selected fungicides on seedling infection from infected seed in growth chamber

Seed treatment with test fungicides significantly reduced seedling infection by *A. pinodes* from infected seeds (Table 3). Carbendazim and iprodione completely inhibited seedling infection while chlorothalonil reduced infection only from 12.5% in untreated seeds to 7.9% which was not significantly different ($p = 0.05$) and the seedling infection was apparent in the untreated and chlorothalonil (Figure 1). The infection on each diseased seedling was slight (1.0) in the case of chlorothalonil and moderate (3.0) in the untreated (Table 3). Nasir and Hoppe (1998) also reported that seed treatment with fungicides reduced incidence of the disease thereby improved emergence and ultimate yield of pea crop. In that report a fungicide containing metalaxyl and thiabendazole formulation was used and was more effective than thiram. Assessment of each plant during seedling studies was strongly recommended by Phillips (1990) who used the same scale repeatedly for *Rhizoctonia* on beans. In this study, the method was found useful to clearly show the effects of *A. pinodes* on seedlings and the effect of fungicides on the inoculum from infected seed, particularly revealing the level of transmission.

Fungicides are obviously useful in disease control practices although some also show phytotoxicity to crops (Maude, 1983). Phytotoxicity of fungicides to field pea seedling during seed dressing was assessed following the recommendation of Hansing (1978) and Sordona (1978) by measuring emergence index (the mean emergence period), emergence percentage, shoot and root (main not lateral) length, and finally shoot and root dry weight. Accordingly, iprodione slightly delayed the emergence period and reduced shoot length shoot and root dry weight while carbendazim and chlorothalonil were not (Table 4). Chlorothalonil was less effective against the target *A. pinodes* in the *in vivo* test, and hence, it should not be considered in the further test. However, carbendazim and iprodione were equally effective against the target fungus (*A. pinodes*) and hence could be a potential candidate for field verification.

In absence of high level of genetic resistance in field pea to the disease, use of fungicide is one alternative. In this regard, fungicide seed treatment may be important means of completely inhibiting or reducing the advancing mycellia during germination and subsequent growth of the plant at early stage either on the seed or in the soil. This early protection of field pea seedling is therefore important and determinant to good yield. Results of these studies clearly showed that some effective fungicides could be used in seed treatment of field pea against Ascochyta blight though subsequent field verifications are essential. Hence, the two most effective fungicides namely carbendazim

Table 3 Incidence and severity index of *Ascochyta* infection on seedlings of field pea raised from fungicide treated seeds in growth chamber at 21 ± 1°C for 25 days.

Treatment	Incidence (%)	Severity index*
Untreated control	12.5	3
Carbendazim	0	0
Iprodione	0	0
Chlorothalonil	7.9	1

* Severity index as a result of scoring in a 0 – 5 ratting scale where 0 = healthy and clean seedlings while 5 = severely diseased seedling leading to seedling death. The values are on the basis of only diseased seedlings (not all seedlings considered).

and iprodione were further studied under field conditions in order to see whether or not seed treatment with these fungicides influence blight development and seed yield of field pea under field conditions.

4. Effects of fungicide seed treatment on *Ascochyta* blight development and seed yield of field pea under field conditions

In field trial, seed treatment with carbendazim and iprodione didn't affect the emergence date after seeding of field pea at both Denbi and Holetta. A mean emergence date of 8.6 days at Denbi and 8.0 days at Holetta were observed. However, variety showed significant difference ($p = 0.05$) in emergence date. Particularly, Tegegnech had a mean delay of about 1 day in emergence at both locations that was significantly different ($p = 0.05$) from LocalH and Mohanderfer (Table 5). This seems due to variety character that relates to

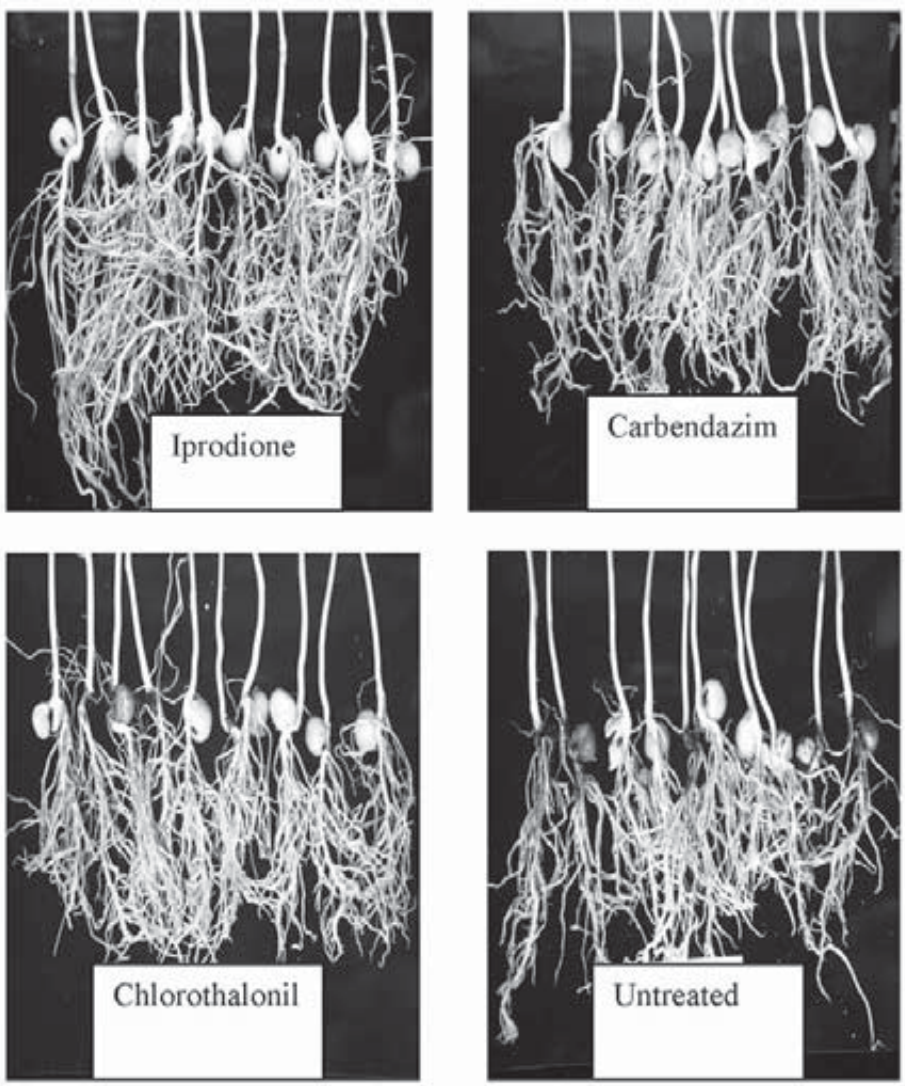

Figure 1 Seedling infection by *Ascochyta pinodes* on seeds treated with iprodione, carbendazim and chlorothalonil and untreated control in growth chamber at 21±1°C 25 days after planting.

Table 4 Effects of fungicide seed treatment on seedling performance of field pea in illuminated growth chamber under 21 ± 1°C for 25 days.

Treatment	Emergence Index	Germination (%)	Length (cm)		Dry weight (g/plant)	
			Shoot	Root	Shoot	Root
Untreated	6.2b*	98a*	35.5a*	8.0b*	1.4ab*	2.4a*
Chlorothalonil	6.2b	99a	35.6a	7.5b	1.4ab	2.5a
Carbendazim	6.1b	100a	37.1a	7.9b	1.5a	2.0ab
Iprodione	6.8a	100a	30.4b	8.7a	1.3b	1.7c

*Means followed by the same letter in each column are not significantly different using Duncan's Multiple Range Test (p = 0.05).

Table 5 Mean emergence time (MET) and emergence count (% of planted seeds) (Stand), Area Under the Disease Progress Curve (AUDPC) for 12 August, 19 August and 26 August (Early) and AUDPC for the whole season (Final) at Denbi and Holetta for the three varieties and three fungicide seed treatments in the 2002 crop season.

Location	Treatment	Emergence		AUDPC	
		MET	Stand	Early	Final
Denbi	**Variety***				
	LocalH	8.3a***	80.8b	167a	1803a
	Mohan	7.9a	89.4a	149a	1941a
	Tegegn	9.6b	71.9a	99b	1656b
	Fungicide**				
	Carben	8.5a	84.1a	150a	1838a
	Iprodione	8.9a	79.9ab	123a	1762a
	Control	8.4a	78.2b	141a	1800a
Holetta	**Variety***				
	LocalH	7.8a	89.3b	96b	1169a
	Mohan	7.4a	90.7b	132a	1326a
	Tegegn	8.8b	92.8a	87b	1189a
	Fungicide**				
	Carben	8.2a	91.7a	111a	1222a
	Iprodione	8.1a	90.7a	102a	1219a
	Control	7.8a	90.4a	101a	1243a

* Variety to be specified as Mohan = Mohanderfer and Tegegn = Tegegnech while **Treatment to be specified as Carben = carbendazim.

*** Means followed by the same letter in each column for the same location and treatment were not significantly different using Duncan's Multiple Range Test (p = 0.05).

its big seed size as larger seeds may require more soil moisture that small size seeds and at early season during planting soil moisture presumed to be limiting.

Emergence count revealed that seed treatment of field pea with fungicides had influenced the seedling establishment of field pea and growth at early stage of the crop development, particularly at Denbi. Carbendazim and iprodione had higher emergence count for the three varieties at this location (Table 5). Particularly Tegegnech had about 6-8% higher emergence than the control at Denbi where there is high blight pressure and only 2-3% higher emergence at Holetta. Ellis and Paschal (1979) reported significantly higher field emergence of pigeon pea when treated with captan and thiram. Kaiser and Hannan (1987) also found that seed treatment with fungicides improved the emergence of chickpea in natural field soil. Thomas and Sweetingham (2003) found that these fungicides increased the field emergence of lupine. Kraft (1982) reported that the main benefit of seed treatment fungicides to pea was the increase of plant stand. Similarly, this enhancement of seedling establishment of the crop observed in this study, may have a substantial effect on the ultimate yield of field pea crop as also reported for many crops by these previous studies.

Seed treatment had suggestive influence on early establishment of Ascochyta disease caused by *Ascochyta pinodes* (teleomorph = *Mycosphaerella pinodes*) on field pea. Carbendazim and iprodione significantly ($p = 0.05$) reduced the incidence of Ascochyta infection at early development period of the crop at both locations (Table 6) while there was no significant ($p = 0.05$) difference due to variety (Table 7). The three varieties tested namely LocalH, Mohanderfer and Tegegnech showed similar trends of Ascochyta incidence at both locations. Incidence increased faster at Holetta than at Denbi. Ascochyta infection did not appear up to the end of third week of July at both locations but, shortly after, the disease started on seedling in the untreated control plots. This is the time when weekly disease assessment started at both locations. At Holetta, the incidence reached about 100% level in two weeks while at Dembi it was in about the same level in about three weeks after the first appearance of the disease (Tables 6 and 7). Mean incidence of Ascochyta

Table 6 Incidence of *Ascochyta pinodes* as influenced by seed treatment fungicides in field experiments at Denbi and Holetta (Ethiopia) in the 2002 crop season after planting on June 21 and 22 in the respective locations.

Location	Fungicide	*A. pinodes* incidence (%) on assessment date of			
		28 July	5 August	12 August	19 August
Denbi	Control	0.08a*	10.43a	18.19a	96.79a
	Iprodione	0.00b	0.18b	1.87b	97.46a
	Carbendazim	0.00b	0.08b	0.08b	96.93a
	Mean	0.039	0.025	7.58	-
Holetta	Control	0.12a	10.44a	97.03a	100
	Iprodione	0.00b	0.98b	96.97a	100
	Carbendazim	0.00b	0.72b	96.57a	100
	Mean	0.04	3.567	96.869	-

* Means followed by the same letter in each column for the same location are not significantly different using Duncan's Multiple Range Test ($p = 0.05$).

infection was 0.03, 3.57, 7.58 and 97.06% for 28 July, 5 August, 12 August and 19 August assessment dates at Denbi, respectively. At this location the incidence sharply increased from 7.58% to 97.12% in just a week time, during 12 August to 19 August while at Holetta the incidence was 0.04, 4.04 and 96.87% when measured on 28 July, 5 August and 12 August, respectively. Similar to Denbi, the incidence increased from 4.04 to 96.87% within a week period during 5 to 12 August at Holetta (Table 6 and 7). This might occurred due to high inoculum of the fungus coming from an external source as the soil was effectively solarized and there was no any fruiting body of the fungus observed on the lesions at that stage. As the effectiveness of seed treatment fungicides does not last long, it might be useful to use other control measures against late infection of *A. pinodes.*

At both locations, fast increases of incidence were recorded from less than 10 to about 100% within a week time, but at different weeks. Neither, pycnidia nor psuedothecia were formed on the lesions of all plants showing disease symptoms at early stage. But after mid-August numerous pycnidia were developed on lesions and not psuedothecia. However, the symptoms of Ascochyta infection were conspicuous and only very few plants died due to the infection. Blight severity was slightly different just before 19 August at both locations and latter there was no difference due to fungicide seed treatment except for the varieties.

Michall *et al.* (1998) reported that reduced seed infection by the same pathogen to be accompanied with reduced blight severity and increased seed yield of field pea. However, Moussart *et al.* (1998) found that infected seeds had high transmission of the disease but remained to only the basal parts of field pea plant that did not influence the blight development later in the crop. This report agrees with that of Xue *et al.*(1996) in there was no significant correlation between percent seed infection and severity of blight in the field. Similarly, in this study seed treatment to reduce the primary inoculum of this pathogen was not associated with blight development latter in the field. Bretag *et al.* (1995a) also found that there was no correlation between seed infection incidence and blight severity at the end and in this study

Table 7 Incidence of *Ascochyta pinodes* as influenced by variety in field experiments at Denbi and Holetta (Ethiopia) in the 2002 crop season after planting on June 21 and 22 in the respective locations.

Location	Variety	*A. pinodes* incidence (%) on assessment date of			
		28 July	5 August	12 August	19 August
Denbi	LocalH	0.03a*	3.23ab	8.16a	97.10a
	Mohanderfer	0.03a	3.30b	6.78a	96.98a
	Tegegnech	0.02a	4.18a	7.80a	97.12a
	Mean	0.03	4.04	7.58	-
Holetta	LocalH	0.02ab	3.90a	97.23a	100
	Mohanderfer	0.00b	3.76a	97.00a	100
	Tegegnech	0.10a	4.48a	96.38a	100
	Mean	0.04	4.04	96.87	-

* Means followed by the same letter in each column for the same location are not significantly different using Duncan's Multiple Range Test (p = 0.05).

reducing the inoculum with fungicide did not influence the blight development in field pea crops.

Seed yield of field pea was affected by seed treatment fungicide before seeding at both Denbi and Holetta being lower at Denbi than Holetta. A mean seed yield of 2.15 t/ha was obtained at Holetta while it was only 0.80 t/ha at Denbi that were significantly ($p = 0.05$) different (Table 8). The seed yield was significantly ($p = 0.05$) different for the three varieties tested at both locations. At Denbi, mean seed yields of 0.55, 0.80 and 1.06 t/ha were harvested for variety LocalH, Mohanderfer and Tegegnech, respectively. But at Holetta, it was 1.93, 2.19 and 2.33 t/ha were harvested for the respective varieties. However, variety Mohanderfer and Tegegnech significantly ($p < 0.05$) out-yielded variety LocalH while there was no difference between them at Holetta. Gorfu (2000) reported higher seed yield of two of these varieties at Denbi than at Holetta, which is contrary to this study. This was clearly seen in the data reported that the difference depended on blight pressure. During previous study, final blight severity was as much as 90% at Holetta and only 58% at Denbi (Gorfu, 2000). But in this study the final blight severity was 63.5% at Denbi and 36.5% at Holetta that is exactly the opposite. And hence, the higher seed yield reported in this study confirm that of the previous study.

The seed yield was also slightly influenced with seed treatment by fungicides at both test locations, Denbi and Holetta (Table 8). At Denbi, mean seed yield of 0.84 t/ha was obtained for iprodione while 0.88 t/ha was for carbendazim. The untreated control gave significantly ($p = 0.05$) lower seed yield of 0.69 t/ha when compared with both the fungicide treated plots. However at Holetta, the difference in seed yield for fungicide treatment was not that high when compared with the untreated plots (Table 8). Mean seed yields of 2.03, 2.21 and 2.21 t/ha were harvested from plots with untreated check, iprodione and carbendazim, respectively. There was no significant ($p = 0.05$) interaction between variety and seed treatment factors at both Denbi and Holetta. As the variances of the two sites were homogeneous, combined analysis was performed to see the overall performance of the seed treatment options. Consequently, the trend of performance was like that obtained at Denbi (Table 9). The three varieties had significantly different seed yields in a trend of Tegegnech > Mohanderfer > LocalH. On the other hand, seed treatment with

Table 8 Mean seed yield of field pea as influenced by variety and seed treatment fungicides in field experiments at Denbi and Holetta (Ethiopia) the 2002 crop season.

Location	Variety	Seed yield (t/ha) for fungicide treatments			
		Carbendazim	Iprodione	Control	Mean
Denbi	LocalH	0.57	0.66	0.42	0.55c*
	Mohanderfer	0.84	0.85	0.73	0.80b
	Tegegnech	1.23	1.02	0.92	1.06a
	Mean	0.88a	0.84a	0.69b	-
Holetta	LocalH	1.92	2.04	1.82	1.93b
	Mohanderfer	2.30	2.19	2.08	2.19a
	Tegegnech	2.41	2.40	2.19	2.33a
	Mean	2.21a	2.21a	2.03a	-

* Means followed by the same letter for variety (in columns) and fungicide (in rows) were not significantly different using Duncan's Multiple Range Test ($p = 0.05$)

Table 9 Mean seed yield of field pea for three varieties and three seed treatment factors in field experiment at Denbi and Holetta (Ethiopia) of the 2002 crop season.

Variety	Seed yield (t/ha) for fungicide treatments			
	Carbendazim	Iprodione	Check	Mean
LocalH	1.24	1.35	1.12	1.24c
Mohanderfer	1.57	1.52	1.40	1.50b
Tegegnech	1.82	1.71	1.55	1.70a
Mean	1.54a	1.53a	1.36b	-

Means followed by the same letter in the last column for variety and in the last row for fungicide were not significantly different using Duncan's Multiple Range Test (P = 0.05).

iprodione and carbendazim increased the seed yield by 12.5 and 13.2%, respectively. Similarly there was no significant interaction between the variety and seed treatment factors at both location and in the combined analysis.

Generally, in this study and previous ones also, seed yield of field pea was dependent on blight pressure and the varieties used. Seed treatment with fungicides improved early establishment of field pea crop, reduced the primary infection of *A. pinodes* and slightly increased the seed yield without affecting the general blight progress throughout the growing season. The seed yield of field pea was increased through an increase in biomass and pod/plant, though the pods/stem was constant in this study. Garry *et al*. (1998) also reported that this pathogen, *A. pinodes*, affects all stages of field pea crop by decreasing plant growth, biomass, and ultimately the seed yield that were mostly reflected on seed weight and number of seeds per plant. Seed treatment alone did not show successful control of blight pressure where foliage treatment was also required to suppress the blight pressure to a satisfactory level. Hence, it would be advisable to use seed treatment options as a component of integrated blight management program in field pea production. Both carbendazim and iprodione can be used against Ascochyta blight caused by *A. pinodes*.

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